Description of Additional Supplementary Files:

Supplementary Data 1: RNAsequencing results. This supplementary data file contains mRNA read counts, DESeq2 comparisons between samples and GO enrichment results.

Supplementary Data 2: ObgE* suppressor mutations. This supplementary data file contains all mutations that were found to provide resistance to ObgE*. This list includes spontaneous suppressor mutations and *lpxA* suppressors that were isolated from the *lpxA* saturation mutagenesis library.

Supplementary Data 3: CRISPRi sgRNA sequencing results. This supplementary data file contains sgRNA read counts and DESeq2 gene-level comparisons between samples.

Supplementary Movie 1: L-form growth of *E. coli* Vector, ObgE or ObgE*. Time lapse microscopy recordings of E. coli pBAD33Gm (Vector), pBAD33Gm-*obgE* (ObgE) or pBAD33Gm-*obgE** (ObgE*) cells as they transition into the L-form state and proliferate.

Supplementary Movie 2: L-form growth of *E. coli* ObgE* in the presence of ROS scavengers. Time lapse microscopy recordings of *E. coli* pBAD33Gm-*obgE** (ObgE*) cells as they transition into the Lform state and fail to proliferate, even in the presence of the ROS scavengers sodium pyruvate, DMSO and MnTBAP.

Supplementary Movie 3: Two-stage L-form lysis in a single cell expressing *obgE**. Time lapse microscopy recordings of a single *E. coli* pBAD33Gm-*obgE** cell as it transitions into the L-form state where it subsequently fails to proliferate and lyses in two distinct steps.

Supplementary Movie 4: Two-stage L-form lysis in a dividing cell expressing *obgE**. Time lapse microscopy recordings of an *E. coli* pBAD33Gm-*obgE** cell that is in the process of dividing as it transitions into the L-form state where it subsequently fails to proliferate and lyses in two distinct steps. The first lysis steps leads to the liberation of two separate and independent entities that subsequently complete the second step of lysis at different times.

Supplementary Movie 5: Two-stage L-form lysis triggered by ObgE* corresponds to first the rupture of the outer membrane and then the loss of inner membrane integrity. By using a blue periplasmic and red cytoplasmic marker, this time lapse microscopy recording of *E. coli* pBAD33Gm-*obgE** L-forms demonstrates that the first step of lysis corresponds to loss of periplasmic content as the outer membrane ruptures and subsequently full cell lysis when the inner membrane integrity and cytoplasm are lost.